UNCLASSIFIED

AD NUMBER

AD836168

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; SEP 1963. Other requests shall be referred to Department of the Army, Fort Detrick, MD 21701.

AUTHORITY

SMUFD D/A ltr, 8 Feb 1972

THIS PAGE IS UNCLASSIFIED

TRANSLATION NO. 893

DATE: Sept 1963

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Translation (French-English) No. T-122-1

MICROBIOLOGICAL DEWAXING WITH PRODUCTION OF Protein and VITAMIN CONCENTRATES

By A. Champagnat, Ch. Vernet, B. Laine, and J. Filosa*

Section IV - Paper 4, - PD 10 France, (1963)

Abstract. The culture of microorganisms on petroleum substrates has been studied with a view of producing protein and vitamin concentrates suitable for animal and human consumption. Owing to the fact that such micro-organisms selectively metabolize paraffinic hydrocarbons, the distillates so treated are thoroughly dewaxed. Usually one part of protein-vitamin concentrates and 9 parts of very low pour point gas oil are produced from 10 parts by weight of heavy gas oil.

The proteins in such concentrates are particularly rich in the amino-acids indispensable to life which are only found in animal proteins. Their use with cereals produces compound foods having a balanced nutritional value. The presence of a high protein of growth vitamins increases their food value.

The profitability of the process is enhanced by the upgrading of the oil fractions as a result of their being freed from wax.

It is shown that the production potential of proteins from petroleum could make good the present world shortage of animal proteins for human consumption in a short space of time and at a competitive price.

INTRODUCTION

In 1957 a microbiological research organisation was set up at the laboratory of Lavera belonging to the Societe Francaise des Petroles BP (The French subsidiary of British Petroleum). Its purpose was to study the action of micro-organisms on hydrocarbonates, and especially on the categories of hydrocarbonates which result from the refining of crude.

The research aimed, and still aims, at developing new means of refining and selection which had not yet led to results usable by the industry. But they quickly made the authors realize the following fundamental facts:

1) Numerous microorganisms live and grow with hydrocarbons as the sole source of carbon and energy. Certain species flourish in the decantation basins of oil refining plants, in the water at the bottom of oil tanks, in the oil-soaked terrains, and even under the asphalted surface of roads. We have especially isolated pseudomonas and certain yeasts. Those micro-organisms are mainly aerobious organisms. They accomodate themselves quite well to raw petroleum substrates, which contain the various classes of hydrocarbons and their impurities.

2) The aerobious micro-organisms which have been studied selectively metabolise essentially straight-chain paraffinic hydrocarbons.

3) Those micro-organisms form a living matter. Therefore they are more or less rich in proteins. Petroleum can thus become a source of proteins, which are the most indispensable element in the diet of man and animals, and the one which they lack most.

These constatations draw the framework of the study which the present communication represents, i.e. the biosynthesis of proteins from petroleum.

It must first of all be recalled that scientific literature regarding the growth of micro-organisms at the expense of hydrocarbons is abundant. The works of J.C. Senez and of his aides regarding the bacterian oxydization mechanism of paraffinic hydrocarbons must especially be mentioned (1).

But, while the previous publications regarding the growth of micro-organisms at the expense of hydrocarbons have had a fundamental, or academical character, the present work proceeds from a decidedly industrial concept. The latter's aim is to achieve, in viable technical and economic conditions, the mass-production of proteins from non-refined parts of petroleum. The de-waxing of those parts is a consequence of fermentation, which represents an evident interest for oil people. It was therefore necessary also to explore its possibilities.

PETROLEUM FERMENTATION

Before proceeding any further, we shall recall some fundamental notions in this new technique which opens for our industry. The micro-organisms represent live matter. Like the plants and the animals, they: -

Only can live in the presence of water.

They need food: carbon, azote, phosphorus, potassium, magnesium, and numerous oligo-elements.

The aerobious microbes breathe oxygen, which means that the combustion of a part of their carbon provides the energy needed for the synthesis of the living cell matter, with discharge of CO_2 and H_2O.

Their growth often requires growth factors such as vitamins, etc.

They reproduce themselves by burgeoning or by sporulation.

Their constitution depends on the conditions and on the medium of culture, and also on their alimentation. It is possible to obtain "fat" cells rich in lipids, or 'lean' ones rich in proteins.

Their growth may be hampered or stopped by poisoning.

They die and then they rot.

It is possible to retard or prevent their putrefaction by cold, by antiseptics or by means of dessication.

Those are the general laws of live, which rule the fermentations in general, and also the Fermentation of Petroleum.

The fermentation industry may do two things:

a) Cultivate micro-organisms in order to harvest them and to use them such as -- that is what is done in our case -- or it can extract from them some valuable products (vitamins, amino-acids, etc.).

b) Separate or extract the products achieved by the micro-organisms in the aqueous culture media. This field is also open to the petroleum industry.

PECULIARITIES RELATIVE TO THE FERMENTATION OF PETROLEUM

We limit ourselves here to the aerobious fermentation.

1. Source of carbon.

Sugars and generally the carbon hydrates which are soluble in water are the source of carbon and energy of the traditional fermentations.

In the petroleum fermentation, the source of carbon and energy lies solely in hydrocarbons, generally normal paraffinic ones.

Petroleum fermentation therefore occurs in a médium with 2 liquid immiscible phases: aqueous medium and hydrocarbons. That is its main difficulty from the point of view of chemical engineering.

p

2. Oxygen needs and yield

The raw formula of the matter which constitutes the cells of the micro-organisms is roughly $C H_2 O$, azote (7 to 15%), phosphorus and other mineral ions being set aside. When they are cultivated on substrate of carbon hydrates of the formula $(CH_2O)_n$ (?), oxygen is needed in order to provide the requirements of energy for the growth which produces CO_2 and H_2O, but there is practically no oxygen fixated on the cell matter. When one starts from paraffinic hydrocarbons of the rough formula $(CH_2)_n$ as substrate, in order to obtain CH_2O, one needs an O atom per CH_2 grouping in order to constitute the matter of the cell. Therefore this matter finally contains in weight 14 parts originating from hydrocarbons for each 16 parts originating from the air. If the reaction occurred without use of energy, one would obtain around 200 parts of cell matter for each 100 parts of metabolised hydrocarbons.

Such is not the case, for one part of the metabolised hydrocarbons is consumed by the respiration of the cell, with production of CO_2 and H_2O and of the energy needed by the syntheses which have occurred.

In practice 100 parts of metabolised paraffinic hydrocarbons produce 100 parts of cell matter and the rest is used as fuel for the biosyntheses.

In the traditional culture of yeasts with sugars as substrate, we obtain only 50 parts of cellular matter for 100 parts of sugar consumed. As a consequence, there is marked advantage to produce cellular matter with hydrocarbons, since one fixes oxygen from the air. But evidently one consumes more of it. One must therefore have a particularly well designed mechanism for the distribution of air in the nutritive medium.

3. Separation and washing.

The traditional fermentation produces an aqueous medium which holds in suspension the cells of micro-organisms. The separation is achieved by means of centrifugation or by filtration. The cells are washed with water once or twice and separated from the aqueous medium by centrifugation or filtration. They are then dried on steam cylinders or by "spray drying".

With hydrocarbons as substrates, even with pure normal waxes (paraffins), there is in the nutritive medium a more or less important quantity of oil which must be completely separated from the cells. This aspect of petroleum fermentation has not been the object of any publication that we know. It is nevertheless of capital importance. We have limited ourselves to study it in the case of the yeasts, which can be separated by centrifugation.

The yeast cells, shaped like an egg and having a few microns of diameter, are the agents of an emulsification of the type of water and oil. The nutritive aqueous medium contains tensioactives (?) which result from the fermentation and which are the agents of emulsification of the type of oil in the water. The result of the antagonistic action of those agents is such that, very often, a substantial part of the aqueous medium, which is limpid and separable by means of a simple decantation. There remains on the surface an emulsion layer which contains the cells. The separation of that emulsion in a centrifuge with three openings is satisfactory, Nevertheless, the yeast cream which is obtained and which contains 10 to 20% of cells in aqueous medium, must again be washed so as to eliminate the traces of hydrocarbons which are still present. The water which is present must also be ridden of the salts which it still contains, as well as of other impurities. Those two objectives are achieved by a series of washings in water, followed by centrifugation.

The final drying has a capital importance, for it conditions the quality of the yeast. It must be carried out at such a temperature that the cells do not surpass a temperature of 100°C., and should reach it for as short a lapse of time as possible.

MICROBIOLOGICAL DEWAXING

A considerable number of micro-organisms can be adapted to grow on hydrocarbons as substrates. Most of them preferably consume straight chain paraffinic hydrocarbons. They seem to ignore cyclic hydrocarbons. The isoparaffins generally are not attacked. The authors, nevertheless, have found exceptions. The following isoparaffins, feebly ramified -- 2 methyltetradecane, 2 methyl-pentadecane, 2 methyl-dodecane, 3 methyl-pentadecane -- are clearly oxidised by certain yeasts, but the reaction is much slower than with paraffins devoid of ramification. 2 methyl-tricosane is barely touched.

As a consequence, from the practical point of view, the micro-organisms which are studied here metabolise completely only with normal paraffins. But the presence of other classes of hydrocarbons does not hamper the process, and that is of capital importance.

It was thus interesting to base a dewaxing process on this selectivity. This process is limited to the consumption of normal paraffins at least until C_{25}, the only realm which was studied. Heavy naphtas, kerosenes, gas oils and even heavy gas oils can thus be dewaxed with considerable improvement in their performance in cold temperatures.

Micro-biological dewaxing of certain heavy gasoils and spindles does not reduce in an equally important fashion their flow point when they contain isoparaffins with a high fusion point which are not metabolized.

The economic importance of dewaxing of most of those fractions (parts), which are not generally dewaxed by traditional methods, lies in the fact that it does not produce paraffins for which it would be difficult to find an adequate outlet. Instead of those paraffins, one produces concentratés of proteins and vitamins of a greater value than that of the paraffins, and corresponding to an enormous potential need.

STUDIES ALREADY CARRIED OUT

Numerous microorganisms - yeasts and bacteria - have been adapted to paraffinic hydrocarbons. A selection was later made for a deeper study of the factors of their culture. The criterium which was adopted was the ease of their separation and of their purification.

In the present state of our knowledge, the yeasts arecto be preferred, but it is not excluded that certain bacteria may be chosen later because of their interesting composition in the matter of food value.

The culture of the selected micro-organisms has been studied on substrates of pure normal paraffinic hydrocarbons and especially on fractions of the atmospheric distillation of crudes from the Middle East and from the Sahara, as well as on certain strongly paraffinic petroleum products.

The main work was done on cultures discontinued up to a scale of 70 liters. This is a convenient method for the study of the parameters of growth. Continued cultures are also proceeding. They allow to draw very important data for the industrial fermentation which must be made in a continuous manner.

RESULTS ALREADY ACHIEVED

1. It has first been established that the mathematical law of the gorwth of micro-organisms in a nutritive medium with a single liquid phase (2) also applies to that growth in a medium of two immiscible liquid phases.

That law is explained by the formula:

$$x_t + x_o \cdot 2^{rt}$$

where: x_o = concentration of cells at the instant o

x_t = concentration of cells at the instant t

r = rate of growth = number of cellular divisions per hour

Time of cellular division = 1/r (generation time).

In our cultures, we easily obtain cellular division times of the order of 4 hours, with wide variations depending on the conditions used. This expression in figures of the speed of reproduction of cells is the essential characteristic of a fermentation. It rules the calculations of the chemical engineers.

2. This nutritive medium of the petroleum fermentation is completely synthetic. In that it differs from the nutritive medium of traditional fermentations from carbon hydrates, derived from agricultural products which often bring in azote, potassium and also some harmful impurities which must be eliminated.

An appropriate nutritive medium must contain, in proportions corresponding to those existing in the cultivated cells, the following elements: mineral azote or organic azote, phosphorus, potassium, magnesium. Those elements are introduced under the form of salts or of compounds, which are as cheap as possible, mainly in the form of chemical fertilysers. Various formulas are possible, which depend on the price of commercial products, on the ability of the micro-organisms to consume them, and on possible incompatibilities.

Furthermore, the nutritive medium must contain the oligo-elements necessary for the growth of the micro-organisms: iron, zinc, copper, manganese, etc. Those oligo-elements exist in the fresh waters and in sea water. The waters which were used in the present study for the preparation of the nutritive medium apparently contained enough of them. The adding of certain among them, which was made during the study, apparently did not result in any improvement of the process.

3. Temperature is a capital factor for the growth. For each micro-organism there is an optima temperature outside of which the time consumed by cellular division (splitting of cells) becomes prohibitive. Those temperatures are all in the neighborhood of 25 to 40°C and mainly towards 30°C.

Since the fermentation is exothermic, a cooling is always necessary.

4. The venue of oxygen is the limiting factor in the cultures of micro-organisms, especially on substrate of hydrocarbons.

In fact the exceptional law of growth does not make it possible to foresee any limit to the concentration of cells in an aqueous medium if one feeds the cells with the needed ions and if no poisoning occurs. The necessary oxygen seems to be consumed by the cells in a state of dissolution in the water of the medium. The limit of the growth is therefore fixed by the diffusion of the oxygen of the air in the medium. In fact, one reaches concentrations in cells (cell densities) of 10 to 25 g. per liter of medium, which is a normal figure in conventional fermentations.

5. Adding of hydrocarbons.

The cells must always have access to normal paraffins which they need. But there must not be too much of them, for the composition of the cells then changes for the worse. On the other hand, if there is a big excess of hydrocarbons, the oil phase hampers the access to the cells for nutritive salts and oxygen, producing a sort of choking.

6. Yields

It is current to obtain cellular densities of 10 to 15 gr. per liter, and sometimes even greater.

The growth yield is expressed as follows in relation to weight:

$$\frac{\text{dry cell matter obtained}}{\text{Metabolized hydrocarbons}} \times 100.$$

It is current to obtain growth yields equal to 100.

The material yield is defined by the following weight relation:

$$\frac{\text{dry cell matter obtained}}{\text{fraction of treated PETROLEUM}} \times 100.$$

This yield depends naturally on the content of metabolized hydrocarbons of the petroleum fraction which is treated, that is its paraffin content. In practice, with the paraffinic gas oils which have been studied, it fluctuates between 8 and 15% and sometimes more.

But for economic calculations the figure 10% must be adopted -- which means that 10 tons of gasoil yield by fermentation 1 ton of dry cellular matter and 9 tons of dewaxed gasoil.

7. Results of dewaxing

One will find below some typical results relative to gas oil with high outflow point.

Tab 1. Results of de-waxing

Origin	Zarzaitine		Irak(?)		Kuwait		Irak(?)		Hassi-Messaoud	
Distillation										
Beginning °C	305	178	224	223	198	244	270	235	204	276
50% ..... °C	327	380	348	366	361	307	328	294	313	335
90% ..... °C	-	-	-	-	-	351	353	365	355	363
final point. °C	351	400	390	400	400	371	367	394	380	379
Paraffin in % per weight*										
before....	13.3	11.6	13.2	14	8.8	7.65	9.5	3.2	4.5	5.6
after.....	0.2	3.4	0.5	1.4	0.9	0.4	0.3	0.6	0.15	0.19
Outflow point °C										
before	+8	+26	+11	+22	+17	-1	+5	-1	+2	+5
after	-25	+3	-24	-16	-20	-25	-34	-37	-37	-34
Material yield in dry cellular matter										
% per weight	12.5	8	13.2	7.8	8.1	15.2	28	8.5	12	9.3

*dosed by cooling in methylene chloride medium.

INDUSTRIAL TRANSPOSITION

The experience acquired in the matter of petroleum fermentation and the studies of chemical engineering which have been carried out make it possible to conceive an industrial unit for a continuous fermentation of petroleum fractions (parts) for the purpose of producing cellular matter for the nourishment of animals and later of man, and, at the same time, to thoroughly de-wax those fractions (parts).

Figure 1 represents a very simplified scheme of such an installation. The previous pages are sufficiently explicit for understanding it.

Fermentator

cream of yeast

drying

Gasoil

recycling nutritive medium

dewaxed gasoil

recycling ~~washing water~~

washing water

dewaxed gasoil

washing water

water moving towards drain

dry yeast moving towards stocking

AIR

nutritive salts

water

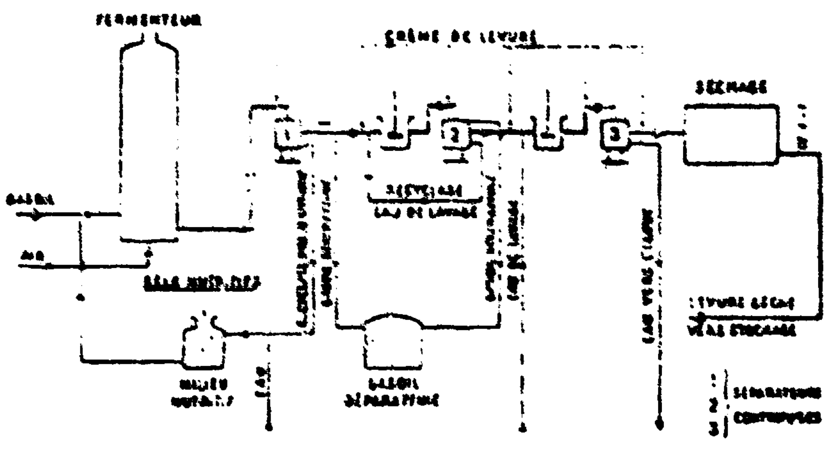
nutritive medium

dewaxed gasoil

1 centrifuge
2 separators
3

Figure 1

Unit of Petroleum Fermentation



In order to ascertain certain elements which are still needed for a final achievement of the industrial stage, a semi-commercial unit is being built at Lavera refinery. That unit will provide information about the chemical engineering of the project. It will make it possible to study (the process) on a sufficiently big scale for transferring on blueprint the knowledge acquired in the laboratory. It will produce sufficient quantities of cellular matter for extensive studies of nutrition on important numbers of animals. Those studies must precede any industrial-scale production.

INVESTMENTS AND MANUFACTURING COSTS

A preliminary study has been made of this subject and it commends the future of this process.

The evaluation has been based on a continuous unit producing 50 tons per day of dry cellular matter. That unit processes 500 tons of gasoil per day and returns to industry 450 tons of dewaxed gasoil per day.

That preliminary economic study indicates that the process is financially interesting and that the eventual profit derives about equally from the production of protein and vitamin concentrates and from the de-waxing of gasoil.

The upgrading as engine fuels of high fluidity gasoils, which are only usable as part of residue fuel oils, must be calculated for each particular case of any refinery.

PROTEIN-VITAMIN CONCENTRATES

This is the name we give to the cellular matter of the micro-organisms cultivated on petroleum substates.

The choice of stocks of micro-organisms and the conditions of their cultivation make it possible to produce protein-vitamin concentrates of different composition. The final selection will only be made thanks to the semi-commercial unit.

Nutrition science provides us with the information necessary to interpret the composition of those concentrates. Man and farm animals need a balanced diet containing well-established proportions of glucids, lipids and proteins, with mineral elements such as phosphorus and calcium, plus a great variety of vitamins.

The deficiency in the single one of those components is enough to cause more or less serious troubles for the physical and mental health of individuals.

The growth of the world population, the geographical diversity of the conditions of life the generalisation of monoculture, are, among many others, the reasons of the under-nourishment of a great part of the human race. The lack of animal proteins is certainly the most widespread form of malnutrition and the one it is most difficult to remedy.

Proteins exist in appreciable quantities in the grains -- wheat, maize, rice, etc. -- which are the basic food, often the only one, of the most part of the poor populations, or of populations unaware of their food requirements. But those proteins are tragically poor in certain amino-acids indispensable to life -- those which the human body cannot produce by synthesis. Only animal proteins contain those amino-acids in sufficient quantity and in required proportions. But it so happens that most micro-organisms also effect that synthesis, with a distribution approaching that which exists among animal proteins.

p

Table 3 gives the global composition of a typical concentrate of proteins and vitamins prepared at Lavera.

Table 2. Investments and Production costs

1. Yearly production (90% utilization........ Production: 50 T/day 16,400 T. (tons)

2. Investment.................................... New Francs: 12.5 million

3. Yearly expenditure	Rate or Price	Quantities	NF X 1000 per year	NF/T produced
Amortization	10%		1,250	76.2
Salaries:				
Operatives.....................	20,000 NF/Year	2 men X 2 jobs	160	
Handling......................	13,000 NF/Year	2 men X 4 jobs	104	21.5
Supervision...................	33%		88	
Upkeep (maintenance) (manpower and supplies)	3% of maintenance		375	22.9
Utilities				
Energy........................	70 NF/1,000 Kwh	18.5×10^6	1,295	
Steam (1 kg)..................	5NF/T	18,500	93	
Cooling water (from sea)......	0.02 NF/T	4.6×10^6	92	142.6
Process water (fresh)	0.24 NF/T	925×10^3	222	
Fuel..........................	90 NF/T	7070	636	
Chemical products (nutrition medium).....	According to price of fertilyzer		3,280	200
Consumed gasoils.........	90 NF/T	16,400	1,476	90
Incidentals.................			550	33.5
Total (without taxes or interest)			9,621	586.7

Table 3.

BP concentrate of vitamins and proteins. Global Composition
(grams per 100 grams)

Moisture	7.03
Total azote	6.92
Proteins	43.6
Lipids	18.5
Glacids (Starch)	21.9
Ashes	4.43
Calcium	0.211
Phosphorus	1.250
Potassium	0.500

Table #4 presents in detaim the distribution of indispensable amino-acids present in this concentrate, in comparison with those contained in a variety of foodstuffs.

Although the interest of the glucids and lipids contained as food is not insignificant, it is the high-grade proteins and the vitamins soluble in water which represent the value of this new food.

Table #4 shows, first of all, that wheat proteins are gravely deficient in lycin. This amino-acid is to be found in sufficient proportion only in animal proteins: meat, fish, milk and yeast. "Lycin is especially the growth limiting factor for the animals. The grain proteins are woefully poor in lycin 3" except in the soya bean. The result is that no grain is without a supplement, a good food for cattle and other herds, or for man.

In the countries where the population suffer of undernourishment in proteins, supplementing grains with lysine is in order[4].

Table 4. Composition of Proteins of various dry foodstuffs

% proteins in dry foodstuff	Wheat flour	Beef	Cow's milk	Dry Torula yeast	BP Protein-Vitamin concentrates
	13.2	59.4	33.1	44.4	43.6
INDISPENSABLE amino-acids					
in g./100 g. proteins	(3)	(3)	(3)	(5)	
Leucine	7.0	8.0	11.0	7.6	7.0
Isoleucin	4.2	6.0	7.6	5.5	3.05
Valin	4.1	5.5	7.05	6.0	8.40
Threonin	2.7	5.0	4.7	5.4	9.10
Methionin	1.5	3.2	3.2	0.8	1.20
Cystin	1.9	1.2	1.9	1.0	0.10
Lysin	1.9	10.0	8.7	6.8	11.6
Arginin	4.2	7.7	4.2	4.1	8.0
Histidin	2.2	3.3	2.6	1.7	8.10
Phenylalanin	5.5	5.0	5.5	3.9	7.90
Tryptophane	0.8	1.4	1.5	1.6	1.17
DEFICIT (8)	LYSIN		CYSTIN + METHIONIN		

The proteins of the new concentrate are related to animal proteins and more closely to those of yeast. In particular, its richness in lysin and threonin is remarkable, but it is deficitary in sulfurated amino-acids: methionin and cystin, which are contained in sufficient quantities in the grains.

The association of grain proteins and of proteins of the protein-vitamin concentrate therefore makes it possible to achieve a sufficiently balanced diet in proteins both for animals and for people.

Beside the indispensable proteins, the presence of a great variety of hydrosoluble vitamins (soluble in water, that is), principally of those of group B, is a very favorable factor (table 5).

Table 5. Vitamins soluble in water

	THIAMIN B1	RIBOFLAVIN B2	NICOTINIC ACID	PANTOTHENIC ACID	PYRIDOXIN B6	COBALAMIN B12
ROLE	SUGARS METABOLISM	DEHYDROGENATIONS OXYDATIONS	TRANSFER OF H2	ACETYLATION SYNTHESIS FAT ACIDS	TRANSAMINATION	FOLIC SYNTHESIS
DAILY RATION in MG	2	3	15	3	2	0.01
EFFECT OF LACK	beri-beri neurites	STOP OF GROWTH	PELLAGRA	STOP OF GROWTH	DERMATITES	PERNICIOUS ANEMIA
FOODSTUFFS						
Beef	1-3	2	40-100	7-21	1-4	
ox-liver	5-10	16	75-275	30-60	5	8
milk	0.3-0.7	1-3	1-5	1-4	1-3	
grains	0.5-7	1-1.5	10-30	5-20	3-6	
oil-cakes	7-14	3-10	10-250	12-50		
dry yeast	2-20 (x)	30-60	200-500	30-200	40-50	
BP PROTEIN-VITAMIN CONCENTRATES	3-16 (x)	75	180-200	150-192	23	0.11

THE AMOUNTS OF VITAMINS ARE EXPRESSED IN mg PER kg. THEY ARE DRAWN FROM THE MAIN REFERENCES (3), (6), (7).

(x) DEPENDS ON THE CONDITIONS OF THE FINAL DRYING

The foods assembled for stock-raising are made up of mixtures of grains or flours of breadstuffs, of oil cakes, etc. to which has been added fish or meat flour in order to complete the ration of high-grade proteins. One must furthermore include growth vitamins, which are often obtained by means of synthesis.

The make-up of the protein-vitamin concentrate shows that it can bring to those compound foods both indispensable proteins and growth vitamins. Its richness in riboflavin (B2) and in pantothenic acid gives it an exceptional value from this point of view.

In this concentrate, as well as in the yeasts, vitamins are associated with other growth factors of unknown consitution, and this compound renders easier the assimilation of proteins by a synergy of action whose effect is superior to that of isolated or synthetic vitamins.[7]

As to the beneficient effect innate in those vitamins regarding human health, it will be recalled that in 1943-1944, in U.S.A., the enriching of wheat flour in B1, B2 and PP was carried out through Government decision. In 1947-1950 in the Philippines the enrichment of rice with B1 brought about the disappearance of beri-beri (3).

The concentrate may suit the nourishment of stock, especially for the raising of young animals: chicken, calves, pigs, etc., by providing high-grade proteins and growth vitamins at a dose of 3 to 5% in the mixed foods.

The introduction of a new protein-vitamin concentrate into human diet naturally does not present much interest in European countries which have abundant meat supplies and would run against their eating habits; but it can bring a notable contribution in high-grade ("noble") proteins to nations of Asia, Africa and South America which suffer of undernourishment for instance

by introducing them into the grain flour, or in the form of extracts to be added to broths.

BIOLOGICAL TESTS

The preceding considerations would be naught but sterile speculation of the proteins-vitamins concentrates were not assimilable without any harm to health.

Biological tests are being carried out on animals since a few months. Those preliminary experiments provide indications of toxicological and nutritional nature which guide us in our task to improve the proteins-vitamins concentrates. The results are already sufficient to persuade us to continue our efforts with confidence.

But that is not sufficient.

When sufficient quantities of concentrates are available, nutrition tests will be made on significant numbers of rats, guinea-pigs, dogs, etc. and that during several generations, with periodical autopsies. Those tests will be directed by the highest science authorities.

Those experiments are also undertaken on farm animals: swine, calves, chicken, etc.

It has already been established that our concentrates have the value of fish flour in the foods prepared for chicken.

But it is only after complete and significantly favorable results that our concentrates will be used commercially.

ECONOMIC CONSIDERATIONS

1. Nutrition of animals.

The industry concerned with the production of foodstuffs for animals is very important. Its gross in the USA is of the order of 3,000 millions of dollars per year.

The providing of "noble" proteins, and especially of growth vitamins for those food compounds may be achieved with the vitamins-proteins concentrate, as it is sometimes achieved with yeast at a dose of 3%

The market for it is considerable and is growing rapidly

For the adding of animal proteins to those foodstuffs, the example of fish four is typical

Peru's production in 1954....................	14,000 T. (tons)
Peru's production in 1962....................	800,000 T.
World production in 1962.....................	2 million tons

The concentrates of proteins and vitamins drawn from petroleum may expect a similar development

Human nutrition.

The minimum daily need in animal proteins is of 30 grams for adults, of 40 to 70 gr. for pregnant women, wet-nurses and children (8). According to those figures the yearly global deficit in animal proteins may be computed at 3 million tons.[9] This corresponds to 15 million tons of muscle meat. It is unlikely that the bringing into cultivation of new lands to be followed by the raising of animal stocks adapted to various climates would achieve such a production within a delay of, say, 40 years. But the current world population of 3 billion will probably double within 40 years; and within 20 years it will be boosted by one billion. That shows the immediate importance of the problem which petroleum may contribute to solve.

With a ton of normal paraffins, which has not previously been separated from the petroleum which it contains, it is possible to produce a ton of protein-vitamin concentrates. The corresponding fractions of petroleum are, to that effect, dewaxed and upgraded if their choice is well done.

For a yearly production of 1,000 million tons of crude oil, one can admit that 700 million tons are paraffinic oil. The production of 7 million tons of proteins-vitamins concentrates, equivalent to 3 million tons of proteins, would consume 1% of those 700 million tons of crude oil. The resources therefore exist, in a most abundant fashion, today.

On the other hand, the contribution of fish flour conveniently de-fatted may constitute a parallel remedy against the lack of animal proteins. Both resources are not superfluous for future needs.

The delay needed for building installations for petroleum fermentation is a matter of 1 to 2 years. Comparing the speed of the production of proteins in those factories with that achieved by nature in the traditional stock-raising farms, one can see that the industrial production is 2500 times faster:

An ox of 500 kg, properly nourished on a grazing land, synthetizes 0.5 kg of protein per 24 hours.[10]

500 kgs of living cells of microorganisms in a continuous fermentator, receiving suitable nutrition in hydrocarbons, azote, phosphorus, potassium and air, must produce, according to our experience, 2,500 kgs. of microorganisms per 24 hours, or 1,250 kgs of proteins.

The muscle meat contains a maximum of 20% of proteins, so that meat proteins cost 5 times the price of meat. Preliminary economic studies show that petroleum fermentation proteins could be produced at a price varying

between 1/5 and 1/30 that of meat proteins at current French prices.

Furthermore, proteins produced in the oil refineries existing in under-nourished countries would be paid in local currency, saving the expense for the import of such products that would be paid in strong currencies.

Until now a source of energy, then of chemical products, petroleum has thus become capable, in competitive conditions, to become a source of higher ("noble") foodstuffs. It can also contribute to solve the number one problem of our time: hunger.

Acknowledgments

The authors thank the management of the Societe Francaise des Petroles BP and of the British Petroleum Company for allowing this publication.

The authors also are especially grateful to Dr. Jacques Senez, of the Center of Scientific Research, for his valuable advice in the fundamental realm.

BIBLIOGRAPHY

1. Senez, J.C. and Azoulay E. - Bacterian degradation of paraffinic hydro-carbons. Annales de l'Institut Pasteur 98 (1960) p. 868-879.

2. Monod J. - Research regarding the growth of bacterial cultures. Hermann, Paris 1958.

3. Jenevrix, M. - Treatise of Biological Chemistry. Presses Universitaires de France, Paris 1957.

4. Rowe, E.E. - Chemical and Engineering News, July 23, 1962 p. 74-82

5. Ingram- An introduction to the biology of yeasts. Pitman, London 1955.

6. Tables of the National Research Council, according to Morrison -- Feeds and Feeding.

7. Gounelle, H. and Cofman S. - Annales de la Nutrition, 1956, vol. X, n° 5-6.

8. Tremolieres J., Serville Y., Jacquot R. - Manual of Human Diet. (Manuel d'Alimentation humaine). Les Editions Sociales Francaises, Paris 1961.

9. The World Food Budget 1962-1965. U.S. Agricultural Department.

10. Thaysen, A.C. - Yeasts. W. Junk, The Hague, 1957.

AUTHORS' BIOGRAPHIES

Champagnat, Alfred / France / Engineer / Ecole Superieure de Chimie of Strasburg / Chief Research Engineer at BP (French subsidiary).

Vernetm, Charles / France / Engineer / Master of Arts and Engineer graduated from the Ecole Nationale Superieure of Nancy (Lorraine) / Director of the laboratories of Lavera refinery of British Petroleum.

Laine, Bernard / France / Engineer / Alumnus of the Ecole Polytechnique / Chief, Research Division at Lavera BP.

Filosa, Jean / France / Engineer / Master of Arts and Chemical Engineer, University of Marseilles / Research Engineer at Lavera, British Petroleum.